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Evolution of a *Wingless* Gene and its Utility for Inferring the Relationships within *Glyphodes* Moths

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The evolution of a nuclear *Wingless* gene was investigated and its utility for inferring the phylogenetic relationship within *Glyphodes* moths was assessed by comparing with other three genes namely, *COI*, *COII*, and *EF-1á*. The results show that the bias of base compositions in *Wingless* (*C*: 0.19) is the lowest among those in *COI*, *COII*, and *EF-1á*. The averages of nucleotide sequence divergences for comparison between groups based on the *Wingless* were the highest. While substitutions in *Wingless* and *EF-1á* genes were not saturated at the divergence of the species groups, *COI* and *COII* genes attained saturation at those levels. The phylogenetic analysis based on *Wingless* solely show that this gene was very useful to resolve the relationships between groups but gave a poor resolution at the specific level, i.e. the relationships within group 1 was not resolved. Combination of all data supports the phylogenetic hypothesis based on morphological data. *Glyphodes* falls into three species groups: group 2 branched off first then followed by groups 1 and 3.

Key words: *COI*, *COII*, *EF-1α*, genes, *Glyphodes*, mitochondria, moth, phylogeny, relationships, *Wingless*

INTRODUCTION

The evolutionary change of morphological characters in a certain group of organisms some times is very complex. Thus, applying of the morphological approach alone might not produce a clear-cut picture of evolutionary history. On the other hand, sequences of nucleotides of genomes provide a much larger amount of phylogenetic information than morphological characters and the evolutionary change of DNA follows a more or less regular pattern. Hence, it is possible to use mathematical model to formulate the change and compare from distantly related taxa.

Mitochondrial and nuclear genes have been used intensively to infer the phylogenetic relationships among groups of insects as have been repeatedly reported by numerous authors (reviewed in Simon *et al*. 1994; Brower & DeSalle 1998). The first has been used to estimate the phylogeny because of the relative technical ease for sequencing from divergent taxa and their special features. Those are the lack of introns, maternal inheritance, absence of recombination events and haploidy (Goto & Kimura 2001). Nevertheless, mitochondrial protein coding genes generally evolve fast and attain saturation rapidly. Therefore, these genes are not always good as phylogenetic markers for certain cases (Liu & Beckenbach 1992). In contrast, nuclear genes are more conserved. This feature is very useful to infer the phylogeny of the distantly-related taxa (Cho *et al*. 1995; Friedlander *et al*. 1998).

Among them, *COI*, *COII*, and *EF-1á* genes have been reported very useful to infer the relationship from species to subfamily level within lepidopteran (Brown *et al*. 1994; Brower & DeSalle 1998; Kim *et al*. 1999; Sutrisno *et al*. 2006). Since those genes showed a rapid rate of substitution in lepidopteran, it holds promise for resolving species level relationships in *Glyphodes* moths.

In this paper, I present the evolution of a nuclear *Wingless* gene by comparing the patterns of *COI*, *COII*, and *EF-1á* from the previous study (Sutrisno 2003; Sutrisno *et al.* 2006) and demonstrate its utility in inferring the phylogenetic relationships within *Glyphodes* moths. I employ simple analytical methods in this paper (maximum parsimony by using equal weighting of all substitutions for tree building, and uncorrected pairwise distance for sequence divergence plots) to illustrate the point I address. I believe that the methods employed here represent the differential dynamics of those gene regions clearly and without bias.

MATERIALS AND METHODS

DNA Sequencing. A total of 14 adult moths were collected from eight localities of Indonesia and Australia (Table 1), by using a light trap, and preserved in absolute alcohol (99.5% ethanol). Technique for the DNA extraction in this paper is following Sutrisno (2003) and Sutrisno *et al*. (2006). For PCR amplification and DNA sequencing of *Wingless* gene, I used a pair primer, namely, Lep WG1, and Lep WG 2 (Brower & DeSalle 1998). The complete sequences of primers were: Lep WG1: 5'-GARTGYAARTGYCAYGGYATGTCTGG-3' and Lep WG 2: 5'-ACTICGCRCACCARTGGAATGTRCA -3'. The sense strand primer of *Wingless* is located at position 1111-1136 in the *Drosophila* sequence and the anti sense strand is located at position 1750-1775.

All PCR reactions were performed in a 50 µl volume containing 5 pM of each primer, 2 mM dNTPs, 2.5 mM $MgCl_2$, 1 x buffer, and 0.25 U of Taq polymerases, by using a Takara Thermal Cycler MP (Takara) in the following PCR conditions:

Table 1. Species examined for the *Wingless* gene sequences

Species	Collection locality	Accession numbers
Glyphodes		
actorionalis (Walker)	Sorong, Papua	AB257147
apiospila (Turner)	Sorong, Papua	AB257140
bicolor (Swainson)	Sorong, Papua	AB257144
bivitralis Guenée	Patinuang, Sulawesi	AB257146
caesalis (Walker)	Menado, Sulawesi	AB257149
conjunctalis Walker	Sorong, Papua	AB257142
cosmarcha Meyrick	Patinuang, Sulawesi	AB257150
doleschalii Lederer	Sorong, Papua	AB257145
flavizonalis Hampson	Sorong, Papua	AB257141
margaritaria (Clerck)	Patinuang, Sulawesi	AB257153
multilinealis Kenrick	Bantimurung, Sulawesi	AB257151
onychinalis Guenée	Bucasia, Queensland	AB257143
pulverulentalis Hampson	Sukabumi, Java	AB257148
stolalis Guenée	Halimun NP, Java	AB257152

one cycle of denaturation at 94 °C for 10 min., followed by 35 cycles, with each cycle consisting of denaturation at 92 °C for 30 sec., annealing at 47 °C for 30 sec., and extension at 72 °C for 1 min. 30 sec. These cycles were completed by final extension at 72 °C for 10 min and the PCR products were purified by QIAquick PCR Purification Kit (Qiagen, USA).

The cycle sequencing was generated under conditions of 35 denaturation cycles at 96 °C for 10 min., annealing at 50 °C for 5 min., and extension at 60 °C for 4 min, and thereafter the products were purified with phenol-chloroform protocol following the manufacture's procedures. The sequencing of the purified PCR product was performed using an ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer) on ABI PRISM model 310 Genetic Analyzer (PE Applied Biosystems). The sequences were aligned using BioEdit Sequence Alignment Editor (Hall 1999).

The sequences of *COI*, *COII*, and *EF-1á* genes used for comparing the pattern of molecular evolution in this study were taken from the DNA Data Bank of Japan (http://www.ddbj.nig.ac.jp) under the accession numbers AB158228-158251, AB158321-158344, and AB158377-158400.

Base Composition Analysis. I used the base frequency's option in PAUP* version 4.0b.10 for 32-bit Microsoft Windows to evaluate the base composition of each sequence and the homogeneity of the base frequency across taxa.

Transition/Transversion Analysis. Transitional and transversional substitutions and transition/transversion (Ts/Tv) ratio were analyzed by using DNA Sequence Analyzer Version 1.00 (Kyukov 1997).

Phylogenetic Analysis. In the phylogenetic analysis with PAUP 4.0b4a (Swofford 2001), I adopted Maximum-Parsimony method with heuristic searches using the TBR branch swapping option and 10000 random addition sequences. The statistical confidence was evaluated by Bootstrap test with 1000 replications (Felsenstein 1985).

RESULTS

Base Composition and Sequence Divergence. Sequences of the 400-bp *Wingless* from 14 species of *Glyphodes* were aligned with no evidence of insertion and deletion. Sequences of the gene have been submitted to the DNA Data Bank of Japan (http://www.ddbj.nig.ac.jp) under the accession numbers AB257140-AB257153.

Table 2 shows the A-C-G-T proportion, and the bias (*C*) was calculated by

$$C = \left(\tfrac{2}{3}\right)\sum_{i=1}^{4}\left|c_i - 0.25\right|,$$

where c_i is the frequency of base *i* (Irwin *et al*. 1991). The bias in *Wingless* and *EF-1á* was low, there were 0.19 and 0.069, but *COI* (*C*= 0.26) and *CO II* (*C*= 0.39) were high. Percentage of A+T in *COI* and *COII* was almost double than in *EF-1á* and *Wingless*.

Interspecific variations in the base compositions in all genes were very low for the total nucleotides. The chi-square test of homogeneity of base frequencies across taxa indicated that there was no significant difference in the frequency of bases between taxa in *COI*, *COII*, *EF-1á*, and *Wingless* (X^2 = 7.80, df = 39, P = 0.99; X^2 = 6.86, df = 39, P = 1.00; X^2 = 2.11, df = 39, P = 1.00; X^2 = 14.08, df = 39, P = 0.99, respectively).

Table 3 shows that the averages of estimated sequence divergence for comparison between groups in *Wingless* were the highest. Whereas in the comparisons species within groups, sequence divergence in *EF-1á* was the lowest.

As shown in Table 4, the informative sites constituted in *Wingless* was the highest (15.5% of 400 bp), whereas in *EF-1á* is the lowest (9.35% of 973 bp). The third-codon positions were most variable but second-codon positions were least variable in all genes.

Figures 1a-d, show the scatter plot of *p*-distance between Ts/Tv ratio and all substitutions in *COI*, *COII*, *EF-1á*, and *Wingless*. The means of Ts/Tv ratio in *Wingless* and *EF-1á* (1.20 and 2.90) was higher than those found in *COI* and *COII* (0.65 and 0.79).

Table 2. Proportion of each nucleotide and bias in *COI, COII, EF-1á* and *Wingless*

	1st-codon	2nd-codon	3rd-codon	Mean
		COI		
A	0.32	0.17	0.48	0.32
C	0.14	0.25	0.04	0.14
G	0.26	0.17	0.09	0.17
T	0.28	0.41	0.46	0.38
Bias (*C*)				0.26
		COII		
A	0.39	0.29	0.41	0.36
C	0.14	0.17	0.03	0.11
G	0.18	0.18	0.01	0.11
T	0.30	0.42	0.57	0.43
Bias (*C*)				0.39
		EF-1α		
A	0.28	0.32	0.13	0.25
C	0.18	0.27	0.44	0.30
G	0.38	0.14	0.21	0.24
T	0.15	0.27	0.22	0.21
Bias (*C*)				0.06
		Wingless		
A	0.23	0.27	0.06	0.19
C	0.27	0.24	0.44	0.31
G	0.32	0.28	0.40	0.33
T	0.19	0.21	0.09	0.16
Bias (*C*)				0.19

Table 3. Uncorrected mean pairwise sequence divergence for *COI*, *COII*, *EF-1á*, and *Wingless* genes

	Mean pairwise divergence (%)			
	Within			Between groups
	Group I	Group II	Group III	
COI				
All data	7.35	7.55	5.92	9.10
1st /2nd/3rd/	0.90/0.09/6.20	0.11/0.32/6.00	1.26/0.10/4.56	1.40/0.2/7.48
COII				
All data	4.72	5.22	5.32	6.51
1st /2nd/3rd/	0.58/0.14/4.00	0.64/0.18/4.40	0.64/0.18/4.50	0.75/0.23/5.53
EF-1á				
All data	2.98	4.55	2.26	5.18
1st /2nd/3rd/	0.30/0.06/2.62	0.25/0.07/4.26	0.14/0.00/2.12	0.43/0.05/4.69
Wingless				
All data	5.05	7.75	3.60	9.57
1st/2nd/3rd/	0.72/0.13/4.20	0.95/0.40/6.40	0.50/0.33/2.83	1.30/0.47/7.80

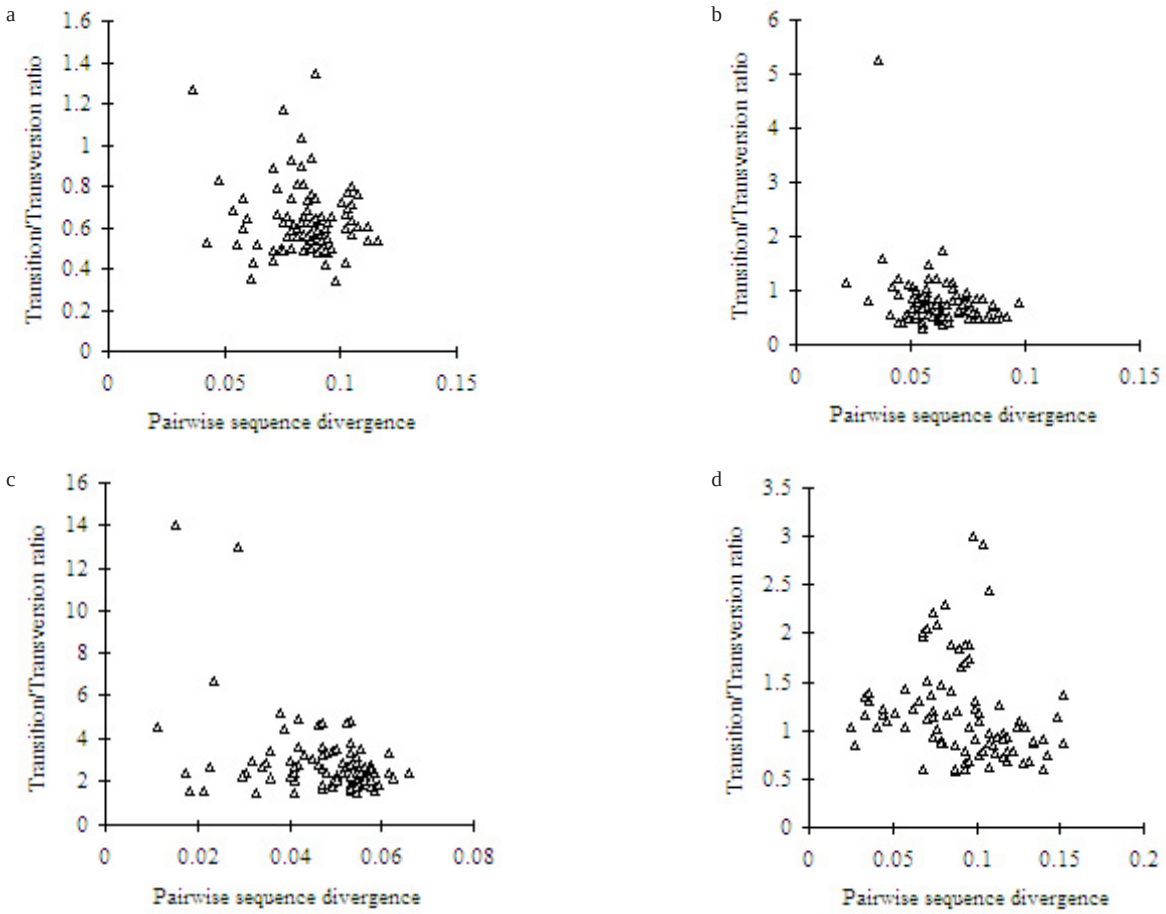

Figure 1. Scatter plots of *p*-distance Transition/Tranversion ratio versus Pairwise sequence divergence. a. In *COI*, b. In *COII*, c. *EF-1α*, d. *Wingless*.

Table 4. Percentage of variable sites across 14 species of *Glyphodes*

	COI	*COII*	*EF-1α*	*Wingless*
Total Positions	686	687	973	400
Variable (%)	27.40	22.85	14.38	25.25
Uninformative (%)	11.85	9.46	5.03	9.75
First (%)	2.47	1.60	0.10	2.50
Second (%)	1.16	0.72	0.20	1.50
Third (%)	8.30	7.13	4.72	5.75
Informative (%)	15.45	13.39	9.35	15.50
First (%)	2.18	1.74	0.82	2.00
Second (%)	0.14	0.14	0.10	0.50
Third (%)	13.11	11.35	8.42	13.00

Figures 2a-d, show the relationships between uncorrected pairwise distances for transitions (Ts) and transversions (Tv). In *Wingless* and *EF-1á* genes, Ts almost linearly increased with respect to Tv and exceeded Tv in all pairwise species comparisons, indicating no saturation of Ts and Tv (Figure 2c, d). The values of linear regressions in *Wingless* and *EF-1á* were $Y = 0.97X$, $R^2 = 0.95$, and $Y = 2.72X$, $R^2 = 0.19$, respectively. In contrast, *COI* and *COII* appeared saturated with transitions when compared between group (Figure 2a, b) and its linear regression was $Y = 1.59X$, $R^2 = -0.25$ and $Y = 0.49X$, $R^2 = -0.49$, respectively.

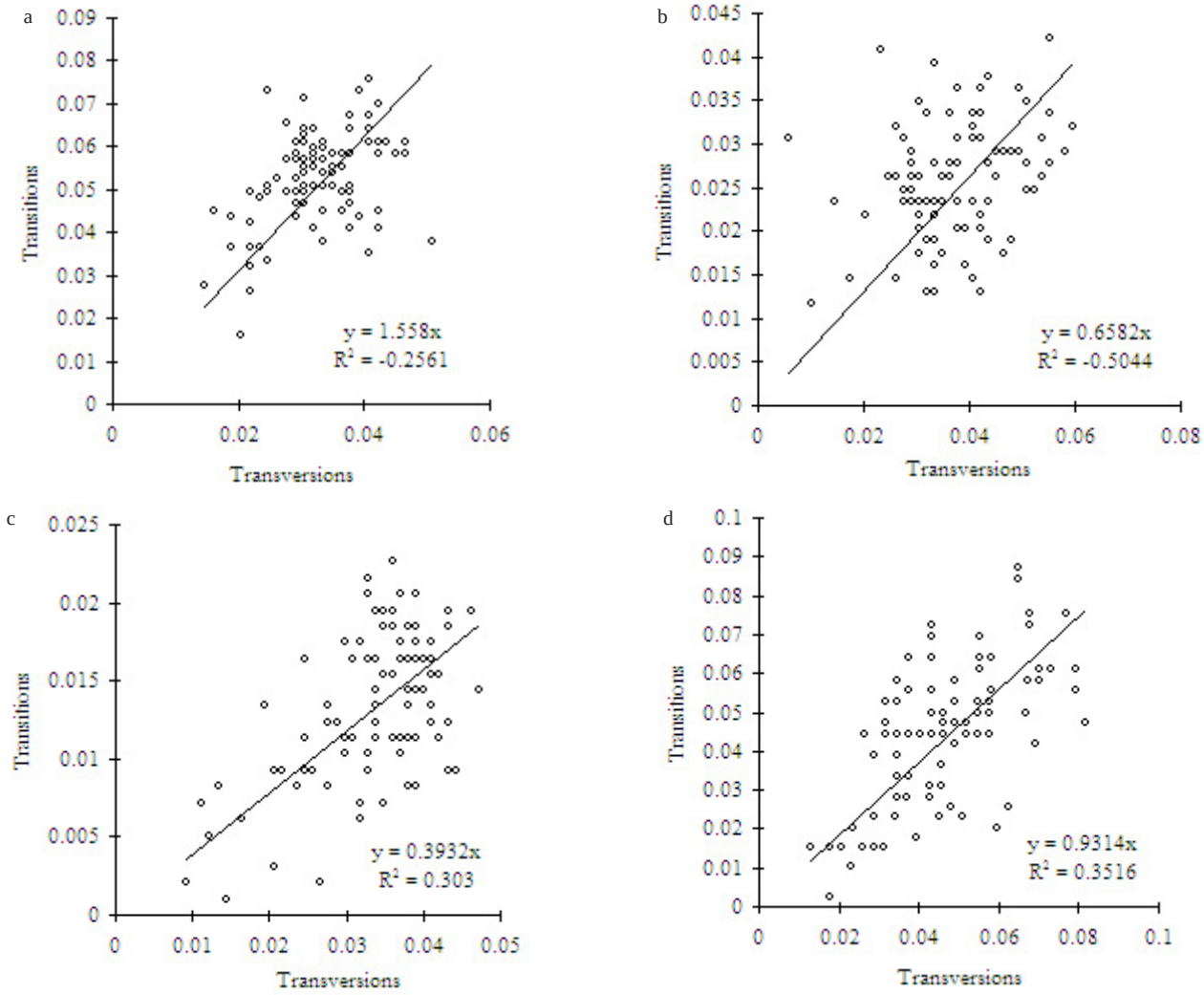

Figure 2. Scatter plots of *p*-distance Transitions versus Transversion. a. In *COI*, b. In *COII*, c. In *EF-1α*, d. In *Wingless*.

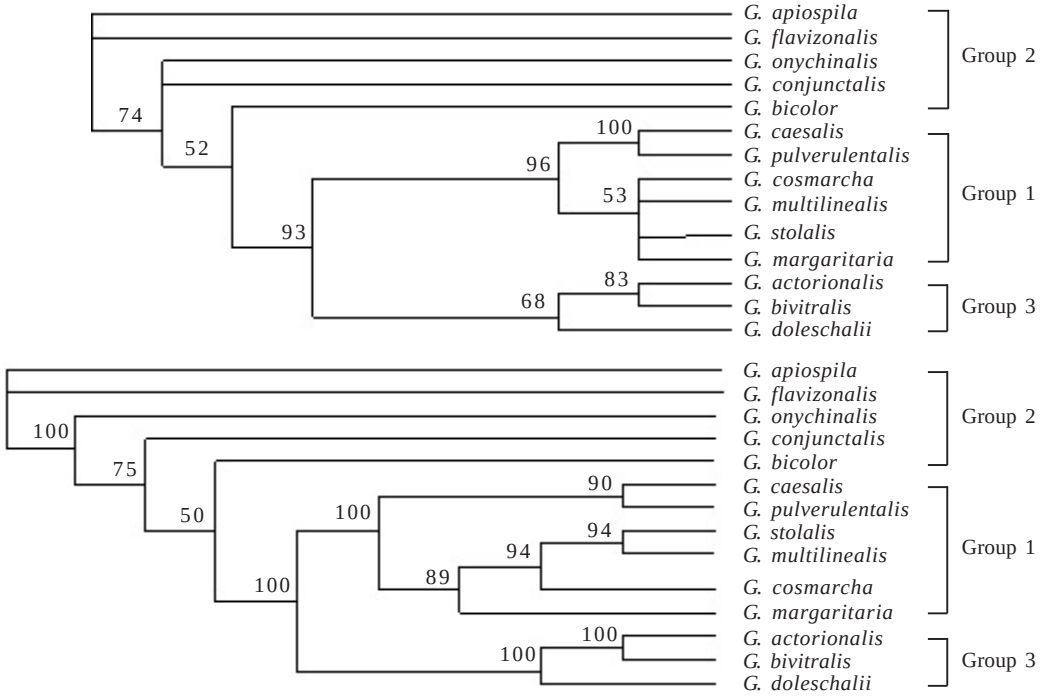

Figure 3. Maximum parsimony tree. a. Strict consensus of the 13 MP trees based on *Wingless*; b. Strict consensus of the 2 MP trees based on pooled data of *COI*, *COII*, EF-1α; and *Wingless*. Bootstrap values with 1000 replicates are shown above the nodes.

Table 5. Summary statistics of cladograms from separate and simultaneous analysis of *COI, COII, EF-1á*, and *Wingless* data

Character sources	No. of bases	No. of trees	TL	CI
Wingless	400	13	190	0.68
All data	2746	2	1173	0.59

TL: Tree length, CI: Consistency index

Phylogenetic Analysis. A heuristic search using all substitutions of *Wingless* resulted in 13 MP trees. The relationships among the species groups were well resolved, but the resolutions among species were poor. When the four data sets were pooled, the analysis resulted in 2 MP trees. The relationships among groups and among species within group almost agreed with the results in the previous study (Sutrisno 2003; Sutrisno *et al*. 2006). *Glyphodes* falls into three species groups: *Glyphodes* group 2 branched off first then followed by groups 1 and 3. The best support was given for the monophyly of group 3. The strict consensus trees based on *Wingless* and pooled data of all genes with bootstrap values for each node are presented in Figures 3a, b. Descriptive tree statistics for parsimony analyses are presented in Table 5.

DISCUSSION

Nucleotide and Amino Acid. The results showed that in *Wingless* and *EF-1á* genes, the first-position nucleotide across all taxa show reduced T (Thymidine) content relative to A, C, and G contents; this possibly explains overall reduced thymidine levels. A similarly reduced T contents in first position was found also in the *Wingless* gene of butterflies (Campbell *et al*. 2000), in the *PEPCK* gene of Lepidoptera (Friendlander *et al*. 1998), in the *Ependymin* gene of fish (Orti & Meyer 1996), in the *EF-1á* gene of *Agrioglypta* and *Talanga* moths (Sutrisno 2005).

Mean base composition among second codon position nucleotides of *EF-1á Wingless* gene show heightened A contents and slightly lowered T and C contents. These results were a similar pattern with those found in the study of *Wingles*s gene in butterflies (Campbell *at al*. 2000) and *EF-1á* in *Agrioglypta* and *Talanga* moths (Sutrisno 2005). This pattern may reflect compositional constraints imposed on second position nucleotides. Specifically, hydrophobic amino acid F, L, I, M, V, A, C; hydropathic indices as defined by Kyte and Doolittle (1982) are never coded for by triplets with A in the second position, whereas hydrophilic amino acids do tend to have A in the second position. Thus secreted proteins with a functional requirement for an overall hydrophilic nature (such as *Ependymin* gene) have high A contents and low TC contents at second positions, whereas membrane spanning proteins have the opposite condition (Naylor *et al*. 1995; Orti & Meyer 1996). Similar requirements may be dictating high A contents in second position nucleotides in *Wingless*, which is a diffusible secreted glycoprotein with a high percentage of hydrophilic amino acid (Couso *et al*. 1994; Perrimon 1996). The moths examined here showed an average hydrophilic amino acid content of 64%, comparable with those found in the butterflies, which is 68% (Campbell *et al*. 2000).

It has been reported that silent sites in mtDNA of vertebrates evolve some 10 times more rapidly than typical nuclear genes. In contrast, silent sites in *Drosophila* nuclear and mitochondrial DNA approximately at the same rate (Powell *et al*. 1986). The results from moths presented here supported the generality of this pattern among panorpoid insects. However, it is clear that further complexities of sequence divergence dynamics emerge from comparison among numerous taxa in phylogenetic context as i.e. sequence divergence of *COI* is higher than *Wingless* for comparison within species group (except for group 3), although for comparison among groups *COI* is lower than *Wingless* (Table 2). This phenomenon also almost similar with those found in the nymphalids that for the distantly-related taxa (above tribal level) the sequence divergence of *COII* is lower than those in *Wingless* (Brower & DeSalle 1998).

The present study reveals that the mean of transitional substitution in *Wingless* and *EF-1á* reflecting a slightly faster overall accumulation of transitions than transversions across all position (Figure 1a). It almost agreed with those found in the study of *Wingless* in Papilionidae which showed 1.3 (Campbell *et al*. 2000). In contrast, in *COI* and *COII*, the value is lower than those found in *Wingless* or *EF-1á* genes (Figure 1b), but it is comparable with results found in the study of mitochondrial *CO I* in *Drosophila* (Goto & Kimura 2001). All these findings support the general view that observed transitions exceed transversions only when recently diverged species or slowly evolving gene are compared (Irwin *et al*. 1991; Simon *et al*. 1994).

The results of this study indicate that transitions in the mitochondrial *COI* and *COII* gene reach a saturation level faster than those found in the nuclear *EF-1á* and *Wingless* genes (Figures 2a, b). This finding is consistent with those found in the study of *Drosophila melanogaster* species group which showed that the transitions in *COI* reached saturation at the level of divergence between subgroups, on the other hand, those in *Gdph* had not reached saturation at those levels (Goto & Kimura 2001). More similar pattern also has been reported in the study of *Eichhorni* group of *Delias* Hübner, in which *ND 5* has saturated faster than *EF-1á* (Morinaka *et al*. 2002). It is, perhaps, because of the A+T rich compositions in the insect mitochondrial genes (Moriyama & Powell 1997).

Phylogeny Analysis. It is not surprising when the phylogeny was inferred from *Wingless* solely show that the relationships among the group 1 and group 3 were well resolved with higher bootstrap supports but this gene fail to show the species relationship within group 1. It seems that this gene obviously contributes great phylogenetic signals to resolve the relationships at the group level as the result of the substitutions of this gene has not reached saturation at those levels of divergence. This finding shows almost a similar pattern with the result found in the study of nuclear *EF-1á* gene in *Hemileuca* which showed that *EF-1á* phylogeny gave better resolution at basal nodes. However, it had shorter branch lengths for many species-level divergence, which resulted in less resolution at the species level (Rubinoff & Sperling 2002). There is no doubt that pooled data given the best resolution and this results support the grouping within the genus

Glyphodes found in the morphological study (Sutrisno 2002, 2003; Sutrisno *et al.* 2006), even was able to show more clearly relationships among them.

Taxonomic Implication on this Study. The practical implication of this study for molecular systematics is clear: *Wingless* gene is very useful to infer the phylogeny but the combination with other data sets appear to be better for inferring relationships species within *Glyphodes* since the topology resulted based on this pooled data showed a better resolution than the separate analysis based on *Wingless* gene solely.

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REFERENCES

Brower AVZ, DeSalle R. 1998. Patterns of mitochondrial versus nuclear DNA sequences divergence among nymphalid butterflies: the utility of *wingless* as a source of characters for phylogenetic inference. *Insect Mol Biol* 7:73-82.

Brown JM, Pellmyr O, Thompson JN, Harrison RG. 1994. Phylogeny of *Greya* (Lepidoptera: Prodoxidae), based on nucleotide sequence variation in mitochondrial cytochrome oxidase I and II: congruence with morphological data. *Mol Biol Evol* 11:128-141.

Campbell DL, Brower AVZ, Pierce NE. 2000. Molecular evolution of the *wingless* gene and its implications for the phylogenetic placement of butterfly familiy Riodinidae (Lepidoptera: Papilionidae). *Mol Biol Evol* 17:684-696.

Cho S *et al*. 1995. A highly conserved nuclear gene for low-level phylogenetics: elongation factor-1á recovers morphology-based tree for Heliothine moths. *Mol Biol Evol* 12:650-656.

Couso JP, Bishop SA, Martinez A. 1994. The wingless signaling pathway and the patterning of the wing margin in *Drosophila*. *Development* 120:621-636.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783-791.

Friedlander TP *et al*. 1998. Two nuclear genes yield concordant relationship within Attacini (Lepidoptera: Saturnidae). *Mol Phylogenet Evol* 9:131-140.

Goto SG, Kimura MT. 2001. Phylogenetic utility of mitochondrial *CO I* and nuclear *Gpdh* genes in *Drosophila*. *Mol Phylogenet Evol* 18:404-422.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nuc Acid Symp Ser* 41:95-98.

Irwin DM, Kocher TD, Wilson AC. 1991. Evolution of the cytochrome b gene of mammals. *J Mol Evol* 32:128-144.

Kim C, Hoshizaki S, Huang Y, Tatsuki S, Ishikawa Y. 1999. Usefulness of mitochondrial COII gene sequences in examining phylogenetic relationships in the Asian corn borer, *Ostrinia furnacalis*, and allied species (Lepidoptera: Pyralidae). *Appl Entomol Zool* 34:405-412.

Kyte J, Doolittle RF. 1982. A simple method for displaying the hydropathic characters of a protein. *J Mol Biol* 157:105-132.

Kyukov K. 1997. *DNA Sequence Analyzer Version 1.00*. Kyoto: Kodansha.

Liu H, Beckenbach AT. 1992. Evolution of the mitochondrial cytochrome oxidase II gene among 10 orders of insects. *Mol Phylogenet Evol* 1:41-52.

Morinaka S, Miyata T, Tanaka K. 2002. Molecular phylogeny of the *Eichhorni* group of *Delias* Hübner, 1819 (Lepidoptera: Pieridae). *Mol Phylogenet Evol* 23:276-287.

Moriyama EN, Powell JR. 1997. Synonymous substitution rates in *Drosophila*: mitochondrial versus nuclear genes. *J M Evol* 45:378-391.

Naylor GJP, Collins TM, Brown WM. 1995. Hydrophobicity and phylogeny. *Nature* 373:565-566.

Orti G, Meyer A. 1996. Molecular evolution of Ependymin and the phylogenetic resolution of early divergence among euteleost fishes. *Mol Biol Evol* 13:556-573.

Perrimon N. 1996. Serpentine proteins slither into the *wingless* and *hedgehog* fields. *Cell* 86:513-516.

Powell JR, Caccone A, Amato GD, Yoon C. 1986. Rates of nucleotide substitution in *Drosophila* mitochondrial DNA and nuclear DNA are similar. *Proc Nat Acad Sci USA* 83:9090-9093.

Rubinoff D, Sperling FAH. 2002. Evolution of ecological traits and wing morphology in *Hemileuca* (Saturniidae) based on a two-gene phylogeny. *Mol Phylogenet Evol* 25:70-86.

Simon C *et al*. 1994. Evolution weighting, and phylogenetic utility of mitochondrial gen sequences and compilation of conserved polymerase chain reaction primers. *Ann Entomol Soc* 87:651-701.

Sutrisno H. 2002. Cladistic analysis of the Australian *Glyphodes* and allied genera (Lepidoptera: Crambidae; Spilomelinae). *Entomol Sci* 5:457-467.

Sutrisno H. 2003. Phylogeny of *Glyphodes* Guenée (Lepidoptera: Crambidae: Spilomelinae) based on nucleotide sequence variation in mitochondrial *CO I* gene: congruence with morphological data. *Treubia* 33:35-42.

Sutrisno H. 2005. Molecular phylogeny of the two closely-related moth genera, *Agrioglypta* Meyrick and *Talanga* Moore (Lepidoptera: Crambidae; Spilomelinae), inferred from nuclear *EF-1á* gene. *Hayati* 12:45-49.

Sutrisno H, Azuma N, Higashi S. 2006. Molecular phylogeny of the Indo-Australian *Glyphodes* and allied genera based (Insecta: Lepidoptera: Crambidae: Spilomelinae) inferred from *COI*, *COII* and *EF-1á* genes. *J Spec Divers* 11:57-69.

Swofford DL. 2001. *PAUP*. Phylogenetic Analysis Using Parsimony (* and Other Methods)*. Version 4.0b10 for 32-bit Microsoft Windows. Massachusetts: Sinauer Ass.